Online Methods

Preparation of mononucleosomes.

Various double-stranded (ds) DNA constructs with different sequences and flanking linker lengths, as shown in Supplementary Fig. 1, were made by PCR and purified by PAGE. When needed, two-nucleotide (nt) single-stranded DNA (ssDNA) gaps were generated in the dsDNA by excision of two adjacent deoxyuridine residues by the Uracil-Specific Excision Reagent (USER, New England Biolabs), which contains a mixture of uracil DNA glycosylase and endonuclease VIII[29]. Prior to USER digestion, the full length dsDNA was constructed by ligation of two separate, PAGE-purified, PCR products with complementary sticky ends made by restriction digestion. Deoxyuridine, 5'-Cy5 and 5'-biotin modifications were incorporated synthetically into PCR primers (Integrated DNA Technologies). For internal Cy5 labelling, PCR primers containing an internal amino modifier (dT C6) (Integrated DNA Technologies) were labeled with a monoreactive Cy5 (GE Healthcare) and purified by reverse-phase HPLC over a C_8 column (GE Healthcare) before use in PCR.

To label the histone H2A protein with the FRET donor, a unique cysteine substitution was introduced to residue 120 of H2A. A Cy3 dye (GE Healthcare) was attached to the cysteine as described previously[23]. The Cy3-labelled and unlabelled H2A were mixed at a ratio of 1:1 together with other histone proteins (H2B, H3 and H4) to form histone octamers. Mononucleosomes were reconstituted from DNA and octamers by salt gradient dialysis and purified by ultracentrifugation over a 10-30% (v/v) glycerol gradient[39]. Without addition of the remodelling enzyme, the FRET values from the nucleosomes remained unchanged for at least one hour after the nucleosomes were anchored to the surface, indicating that the nucleosomes were stable during this time scale.

Preparation of ACF

The procedure to prepare ACF was previously described[23,40]. Briefly, HA-Acf1 and flag-SNF2h were individually over-expressed in Sf9 cells using a baculovirus expression system. Excess HA-tagged Acf1 extract was mixed with flag-tagged SNF2h extract, and the ACF complex was purified using M2-affinity chromatography[40]. For experiments that directly monitor the binding of ACF to nucleosomes, we labelled the ACF complex with the Alexa 488 dye. Prior to M2 elution, the resin was washed with labelling buffer (20% glycerol, 20 mM HEPES pH 7.0, 0.2 mM EDTA,

100 mM KCl, 1 mM Benz HCl, 1 mM TCEP) followed by addition of Alexa 488 maleimide (Molecular Probes) to 100 μM final concentration. After 30 min at 4°C, the labelling reaction was quenched with 80 mM βME for 15 min. The resin was washed extensively with wash buffer (20% glycerol, 20 mM HEPES pH 7.9, 0.2 mM EDTA, 100 mM KCl, 1 mM Benz HCl, 1 mM DTT) and the protein was then eluted. Stoichiometry of Acf1 to SNF2h was confirmed by SYPRO staining.

Single-nucleosome FRET measurements.

Quartz microscope slides were coated with methoxy poly(ethylene glycol) (PEG, Nektar Therapeutics or Laysan Bio Inc), biotin-PEG (Nektar Therapeutics or Laysan Bio Inc) and streptavidin as described previously[25]. The biotinylated nucleosomes were then linked to the streptavidin-coated slide surface via a biotin-streptavidin linkage. The donor and acceptor fluorescence signals from the surface-anchored nucleosomes were excited with a 532 nm Nd:YAG laser (Cyrstal Laser) in the total internal refection geometry and fluorescence emission from Cy3 and Cy5 was detected with a 60x water immersion objective (Olympus), filtered with a 550 nm long-pass filter (Chroma Technology), spectrally spilt by a 630 nm dichroic mirror (Chroma Technology), and imaged onto two halves of a CCD camera (Andor iXon 897 or iXon^{EM}+ 888)[25]. Unlabelled ACF was added to the surface-anchored nucleosomes together with ATP to induce remodeling. In experiments that monitored both the binding of ACF and the remodeling of nucleosomes, ACF labelled with Alexa 488 was added to the nucleosomes. Alexa 488 and Cy3 were excited by alternating 488 nm argon ion and 532 nm Nd:YAG laser lines, respectively.

Since the FRET donor Cy3 is attached to the H2A subunit of the histone octamer, the presence of two H2A subunits in each octamer led to a heterogeneous population of nucleosomes with three different labelling configurations: 1) donor on the H2A subunit proximal to the acceptor, yielding a high FRET level, 2) donor on the distal H2A, yielding a lower FRET level, 3) donor on both H2A subunits, yielding an intermediate FRET value. For example, three distinct peaks centred at FRET = 0. 88, 0.75 and 0.58 were observed in the FRET distribution of the nucleosomes with exit linker length *n* = 3 bp (Fig. 1b). In this work, we focus our analyses on nucleosomes containing a single donor on the proximal H2A. In the FRET histogram analysis, the histograms were fit with three Gaussian peaks and the peak with the highest FRET value was selected. In the FRET trace analysis, traces starting with a mean FRET > 0.75 and exhibiting a single donor bleaching step were selected. This selection process allowed us to include the entire population of nucleosomes with a donor on the proximal H2A without

contamination from nucleosomes with donor on both H2A subunits, which exhibit two donor bleaching steps. Such selection process indeed resulted in a single population of nucleosomes with FRET centred at 0.88 (Supplementary Fig. 2e). Similar selection criteria were used for nucleosomes with exit linker length *n* = 6 bp, 0 bp and -3bp: Traces starting with a mean FRET larger than a threshold value (0.6 for n = 6 bp, 0.75 for n = 0 bp and n = -3 bp) and exhibiting a single donor bleaching step were selected for further analysis. To generate the calibration curve in Fig. 1c, nucleosomes with *n* = 3, 6, 8, 10, 11, 13, 18 and 23 bp were used. The FRET distributions in each case were fit to three Gaussians and the peak with the highest FRET value was selected, except for the *n* = 18 or 23 bp nucleosomes, which exhibited only very low FRET values that appeared as one peak. In these two latter cases, the FRET distribution was fit to a single Gaussian function to extract the peak position.

In experiments where buffer exchange was used to add or remove ACF and ATP, the sample chamber was infused with new buffer using a syringe pump (KD Scientific). To ensure complete buffer exchange within the sample chamber (~20 μL), a large excess of the new buffer was flown through the chamber upon infusion. In the case of ACF and ATP addition, 300 μL or ~15 chamber volumes of new buffer was infused. In the case of ACF removal, 600 μL of new buffer was infused. The dead time for buffer exchange was measured to be 1.3 s.

Nucleosomes were imaged at 30°C in a buffer consisting of 12 mM Hepes, 40 mM Tris pH 7.5, 60 mM KCl, 0.32 mM EDTA, 3 mM $MgCl_2$, 10% glycerol, 0.02% Igepal (Sigma Aldrich), an oxygen scavenging system (10% glucose, 800μg/mL glucose oxidase, 40μg/mL catalase) to reduce photobleaching, 2mM Trolox (Sigma) to reduce photoblinking of the dyes[41], and 0.1 mg/mL BSA (Promega) to prevent non-specific sticking of nucleosomes and ACF to the surface.

Estimate of the characteristic oscillation time, the translocation speed and the total travelling distance of centre-positioned nucleosomes.

The characteristic FRET fluctuation time (τ) of the centre-positioned nucleosomes in the presence of ACF and ATP was derived using autocorrelation analysis. Briefly, the autocorrelation function at each condition was constructed from ~100 FRET time traces. The characteristic time τ was derived as the decay constant from single exponential fitting of the autocorrelation function.

To estimate the cumulative distance (not net distance) of nucleosome translocation on DNA induced by a bound ACF complex as shown in Fig. 4c, FRET traces during the period after removal of ACF from the solution but before dissociation of the bound ACF from the nucleosome were divided into unidirectional segments of monotonically changing FRET. The

FRET change in each segment was converted into the number of base pairs translocated by comparison with the calibration curve shown in Fig. 1c. The results from all segments of a trace were summed to give the total distance travelled by the octamer. The following reasons make the estimated total distance an approximate, lower bound estimate of the cumulative distance travelled by the histone octamer: (i) Only unidirectional segments with a FRET change greater than 0.3 were selected for the sum to avoid counting noise in the FRET signal. To characterize noise, FRET fluctuations from the nucleosomes in the absence of ACF were analyzed and the probability of observing a FRET change greater than 0.3 in the absence of ACF was found to be less than 0.002. Therefore, 0.3 is a rather conservative threshold for nucleosome translocation, leading to an underestimate of travelling distance; (ii) The slope of the calibration curve in Fig. 1c was used to estimate distances from changes in FRET, but as the FRET value gets near 0 or 1, it saturates and becomes an insensitive measure of distance, also causing an underestimate. The possibility that the DNA trajectories within the nucleosome or on the entry side could differ from that of the exit linker DNA may give additional error in the distance estimate. To estimate the speed of octamer translocation, the distance change within each unidirectional segment determined above was divided by the duration of the segment. To determine the number of times a nucleosome switched its direction, only events switching from a decreasing trend to an increasing trend in FRET were counted, as the switching from an increasing trend to a decreasing trend could be due to the FRET acceptor on the DNA passing by the FRET donor on the octamer without switching direction. Therefore, the estimated number of switching times is also an underestimate, as some of the latter type of switching events may also represent real directional switching of the nucleosome translocation.

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